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(54) Vectorized therapeutic agent.

(57) A therapeutic agent, more particularly one that is intended for the treatment of cancer tumors, comprising,associated in the same particular vehicle, substance having a cytotoxic effect, such as an anthracycline like mitoxantrone or doxorubicin, for example, and at least one substance that inhibits multidrug resistance (MDR), such as an alkaloid like quinine, quinidine, cinchonine, cinchonidine, or alternatively amiodarone or verapamil, for example. Preferably, the said substances are associated within the same liposome.

Description:

The object of the invention is a new therapeutic agent, more particularly a therapeutic agent intended for the treatment of cancer tumors presenting the phenomenon of multiple resistance to anticancer agents, as well as a pharmaceutical compound containing the said therapeutic agent.

Another object of the invention is a process which makes it possible to intensify the efficacy of cytotoxic substances used in the treatment of cancer tumors presenting the phenomenon of multiple resistance to anticancer agents (multidrug resistance).

The phenomenon of multidrug resistance is known and is not limited only to anticancer agents. Today, various explanations are advanced to help us understand the mechanisms or sites involved. As regards the behavior of cancer tumor cells, it has been possible to demonstrate several distinct resistance systems: For example, concerning the permeability of the cell membrane, the intervention of a specific glycoprotein (P-gp) is recognized, but it is admitted that other protein factors could be involved.

This phenomenon requires a multiple or even complex approach, which makes it all the more difficult to find appropriate solutions.

The use of cytotoxic substances or drugs in the treatment of cancer tumors comes up against several obstacles. Most drugs used for this purpose present a toxicity which is primarily intrinsic, rather than distinctive, which is the source of harmful side effects; on the other hand, this intrinsic toxicity means that the quantity of them that can be administered to the patient is limited and, in many cases, it is insufficient to achieve the desired activity at the level of the tumors.

Various means have already been proposed to overcome such obstacles, such as vectorization of the cytotoxic substance, for example by incorporating it into a liposome. However, in such a case even if the intrinsic toxicity of the cytotoxic substance is temporarily masked and thus has limited side effects for the patient, its activity at the tumor site is not necessarily reestablished.

Moreover, however little the tumor cells undergoing this treatment present the phenomenon of multidrug resistance (multiple drug resistance, MDR), innate or acquired, the cytotoxic substance loses almost all its efficacy.

Today several substances are known which develop an MDR inhibiting activity *in vitro*: For the most part these are polycyclic, non-cytotoxic substances which generally have a hydrophobic character, such as alkaloids. Their joint use in a cytotoxic drug appears satisfactory in several cases, with the phenomenon called MDR being significantly inhibited at the cellular level so that the said drug can play its full role.

However, the situation is completely different if one attempts to transfer such observations to a living organism. The MDR-inhibiting substances, alkaloids or others, also have an intrinsic toxicity which limits their administration to a threshold (serum level) at which the activity that is sought (inhibition of MDR) is also lost. Moreover, whether the cytotoxic substance be administered in free form or in a vectorized form, for example in a liposome, an important limiting factor which has been observed is that there is a concomitant increase in the intrinsic toxicity of the cytotoxic drug resulting from a significant change in its pharmacological distribution in the organism, which is due precisely to the influence of the inhibiting substance that is used. In effect, faced with such difficulties, the person skilled in the art finds himself particularly deprived of means of *in vivo* treatment of cancer tumors presenting the phenomenon of multidrug resistance (MDR).

The invention has the merit of solving the problem presented above in an original and particularly effective way. Its object is a therapeutic agent comprising, associated in the same particular vehicle, a substance having a cytotoxic effect and at least one non-cytotoxic substance that inhibits multidrug resistance (MDR). Using this new means, the invention makes it possible to act effectively along two distinct axes. This noticeably reduces the toxicity of the active agents that are used, that is the anticancer agent (which is cytotoxic) and the inhibiting substance; on the other hand, the choice of an appropriate particular vehicle makes it possible to provide better targeting of the therapeutic agent to the site being treated, in this case the cancer cells. Moreover, depending on the case, the particular vehicle can play a purely passive role and thus constitute a slow-release formulation.

According to the invention, the particular vector or vehicle that is used can be a microparticle or microcapsule, or a nanoparticle or a nanocapsule, for example a nanosphere. An advantageous candidate for such a vehicle is a liposome, for example a so-called stealth liposome.

Also according to the invention, the cytotoxic substance used is one of a series of substances which share the common feature of being susceptible to the phenomenon of MDR: For the most part these are hydrophobic substances which share the common feature of a positively charged nitrogen group. Cytotoxic substances which can be used according to the invention are Vinca alkaloids, anthracyclines or analogous products, epipodophyllotoxins, or antitumor antibiotics, for example. It is preferable for the cytotoxic substance to be chosen from vincristine, vinblastine, vindesine, vinorelbine, doxorubicin, deoxydoxorubicin, tetrahydropyranyladriamycin, epidoxorubicin, aclacinomycin, demethoxydaunorubicin, daunorubicin, *m*-AMSA, mitoxantrone, bisantrene, plicamycin, actinomycin D, puromycin, etoposide, teniposide, emetine, ethidium bromide, cytochalasin, colchicine, and Taxol®. The acylated derivatives or esters of these compounds could also be mentioned.

According to the invention, a cytotoxic substance such as mentioned above is associated within the same particular vehicle, such as a liposome, with a non-cytotoxic substance that inhibits MDR and whose effect has previously been observed *in vitro*, or *in vivo* in certain cases, but with the limiting factors presented above.

[Translator's note: In the following paragraph and elsewhere, the meaning of "SDB" in "sdb-éthylène diamine" is unknown.]

According to the invention, substances which can advantageously be used for this purpose are amiodarone, quinine, quinidine, cinchonine, cinchonidine, verapamil, cyclosporine A, the cephalosporins, biperiden, lidocaine, chlorpromazine, pentazocine, promethazine, canrenoate potassium, amitriptyline, propranolol, demethoxyverapamil, diltiazem, thioridazine, trifluoperazine, chloroquine, sdb-ethylene diamine, reserpine, tamoxifen, toremifene, hydrocortisone, progesterone, salbutamol, and their acylated derivatives or esters.

As indicated, it is possible to use, within the same vehicle, one or several substances which inhibit MDR. Particularly interesting results were obtained *in vivo* using liposomes incorporating adriamycin or mitoxantrone associated with quinine or cinchonine. The use of two distinct inhibiting substances can be advantageous when the purpose is to inhibit distinct cellular sites or distinct mechanisms of MDR.

The molar ratio of the cytotoxic / inhibitor can vary considerably according to the desired effects, according to the nature of the chosen cytotoxic agent and inhibiting substance, and also according to the chosen vehicle, or even the mode of administration of the vectorized therapeutic agent.

From the perspective of structure, at the level of a particular vehicle such as a liposome, for example, various situations can be envisaged. In a first case, the substance having a cytotoxic effect and the inhibiting substance are both at least partly incorporated in the membrane of the particular vehicle.

In another case, the inhibiting substance can be found in the empty space of the particular vehicle, and the substance having a cytotoxic effect is fixed to the membrane of the said vehicle. It is for the person skilled in the art to choose the most appropriate structure, depending on the nature of the vehicle and the effects that are sought.

The invention makes it possible to propose an appropriate therapeutic treatment for many cancer tumors presenting various degrees of multiple resistance to anticancer agents (MDR). In this connection it is possible to mention, among others, acute myeloblastic leukemia, acute lymphoblastic leukemia, neuroblastoma, small cell lung cancer, ovarian carcinoma, malignant non-Hodgkin's lymphoma, and diffuse plasmacytoma. These are cancers which present an MDR that is induced in response to treatment with a cytotoxic agent.

It is also possible to treat cancers which present an MDR that is innate or at least has cancer cells that are characterized by the presence, before any treatment, of a relatively high level of the gene corresponding to P-gp (MDR 1). These are, for example, adenocarcinoma of the colon, renal adenocarcinoma, adrenocortical carcinoma, pheochromocytoma, pediatric sarcomas, and secondary leukemia. However, this list is not exhaustive.

Another object of the invention is a pharmaceutical compound, more particularly one which is intended for the preventive or curative treatment of cancer tumors which develop the phenomenon of MDR, comprising, as its active ingredient, a therapeutic agent such as defined above. Such a compound could be, for example, in the form of a suspension that can be administered via the parenteral

route, or also in the form of a gel which can be administered via the intraperitoneal route. Of course such forms of administration are not restrictive.

More generally speaking the invention consists of perfecting an original process which makes it possible to increase to a significant extent the activity of a cytotoxic substance which it is desired to use in the treatment of cancer tumors which are developing the phenomenon of MDR. The said process comprises the association of the said cytotoxic substance, within the same particular vehicle, such as a liposome, with at least one non-cytotoxic substance that inhibits multidrug resistance (MDR). The examples below will illustrate the invention in a more detailed manner. In no case are these examples restrictive.

a. *In vitro* experiments

For demonstration purposes, first an *in vitro* test was performed of quinine's potentiation of the antitumor activity of mitoxantrone (MXN).

On D0, first rat colon tumor cells DHD/K12/TRb were implanted in the wells of a culture plate. This cell line is recognized as developing the phenomenon of multiple resistance to anticancer agents (MDR). On D1, the non-confluent cells were treated using progressive concentrations of MXN in the presence of quinine in a fixed-dose solution for a period of 84 hours. Cell survival was analyzed using a methylene blue test.

The results obtained, which are shown in Fig. 1, demonstrate an *in vitro* potentiation of the antitumor activity of MXN under the influence of quinine.

An identical procedure was followed using cinchonine, cinchonidine, and verapamil, respectively and comparable results were obtained. It is confirmed that, *in vitro*, these substances exercise an MDR inhibiting effect.

b. *In vivo* experiments

The model used in this case is peritoneal carcinomatosis of colonic origin, induced in BDIX syngenic rats. The induction was performed by injecting the rats with DHD/K12/TRb cells on D0.

The subjects were divided into 4 groups of 3 rats each, with the treatment beginning on D3 as follows: At H-1 each rat, except for the control rats, receives an intramuscular injection of quinine (100 mg/kg), with the MXN treatment beginning on D0 and being administered by intraperitoneal injections of the indicated doses.

Group 1: 3 mg of free MXN/kg
Group 2: 3 mg of MXN in liposomes/kg
Group 3: no MXN treatment
Group 4: controls

On D26, the rats in the 4 groups indicated above were sacrificed, and autopsies were performed to examine the different stages of development of the peritoneal carcinomatosis.

The evaluation criteria define 4 distinct classes.

Class 0: Absence of macroscopically visible tumor nodules in the peritoneal cavity;
Class 1: Presence of a few small tumor nodules having a diameter of 1 mm, no involvement of the parietal peritoneum;
Class 3: Diffuse peritoneal carcinomatosis, massive infiltration of the mesentery, omentum, and the parietal and diaphragmatic peritoneum (+ hemorrhagic ascites).

The observations made after the autopsy allow the following conclusions to be drawn: Groups 3 and 4 present a class 3 carcinomatosis, and all subjects are living on D26. Group 2 presents a class 2 carcinomatosis, and all treated subjects are living on D26. Group 1 also presents class 2 carcinomatosis, but 2 of the 3 subjects died prematurely.

It can be deduced that the antitumor activity of MXN is at least conserved, but in the case of the free MXN example (Group 1), the general toxicity developed by the pair MXN / quinine goes beyond the maximum permissible threshold, causing the premature death of 2 of the 3 rats.

c. Therapeutic agent according to the invention

The same carcinomatosis model as before is used to demonstrate the efficacy of the treatment.

c.1. First, the usual techniques were used to prepare liposomes containing quinine and mitoxantrone (MXN) in a mass:mass ratio of 30:1.

The rats in which peritoneal carcinomatosis was first induced on D0 were divided into 4 groups of 5 rats each and underwent the following treatment on D1.

Group 1: liposomes containing MXN and quinine in a dose of 2 mg of MXN/kg and 60 mg of quinine/kg.
Group 2: free MXN (2 mg/kg) + free quinine (60 mg/kg).
Group 3: liposomes containing quinine (60 mg/kg).
Group 4: controls.

The treatment on D1 was performed by intraperitoneal injection. On D34 the rats were sacrificed, and then autopsies were performed as described above. It was

observed that Groups 3 and 4 presented a class 3 carcinomatosis, and all subjects were living on D26.

As for Group 2, the carcinomatosis observed is of class 0, but only one rat out of 5 survived to D34.

As for Group 1, the carcinomatosis that developed is of class 1, and all the subjects were living on D34.

These results are illustrated by Fig. 2, 3, 4, and 5, which are put together on a single sheet.

c.2. The usual techniques were used to prepare liposomes containing quinine and doxorubicin (DXR) in a mass:mass ratio of 80:1.

The rats in which peritoneal carcinomatosis was first induced (D0) were divided into 4 groups of 5 rats each and underwent the following treatment on D1.

Group 1: liposomes containing DXR and quinine in a dose of 1 mg of DXR/kg and 80 mg of quinine/kg.
Group 2: free DXR (1 mg/kg) + free quinine (80 mg/kg).
Group 3: liposomes containing quinine (80 mg/kg)
Group 4: controls.

The treatment on D1 was performed by intraperitoneal injection. On D34 the rats were sacrificed, and then autopsies were performed as described above. It was observed that Groups 3 and 4 presented a class 3 carcinomatosis, and all subjects were living on D34.

As for Group 2, the carcinomatosis observed is of class 0, but only one rat out of 5 survived to D34.

As for Group 1, the carcinomatosis that developed is of class 1, and all the subjects were living on D34.

c.3. Exactly the same procedure was used as indicated above, but quinine was replaced by an equivalent quantity of cinchonine.

Group 1: liposomes containing DXR and cinchonine in a dose of 1 mg of DXR/kg and 80 mg of cinchonine/kg.
Group 2: free DXR (1 mg/kg) + free cinchonine (80 mg/kg).
Group 3: liposomes containing cinchonine (80 mg/kg)
Group 4: controls.

The treatment on D1 was performed by intraperitoneal injection. On D34 the rats were sacrificed, and then autopsies were performed as described above. It was observed that Groups 3 and 4 presented a class 3 carcinomatosis, and all subjects were living on D34.

As for Group 2, the carcinomatosis observed is of class 0, but only one rat out of 5 survived to D34.

As for Group 1, the carcinomatosis that developed is of class 1, and all the subjects were living on D34.

On the basis of the experiments listed above, it is easy to see that the inhibiting substances that were tested potentiate the anticancer activity of cytotoxic drugs such as mitoxantrone and doxorubicin. Moreover, once associated in a particular vehicle such as a liposome according to the invention, the said inhibiting substances and the said anticancer agents turn out to be significantly less toxic than when the substances are administered in free form.

c.4. On D0, each rat received an intraperitoneal injection of 1,000,000 DHD/K12/TRb cells to induce, in these subjects, the peritoneal carcinomatosis of colonic origin which was used before as an experimental model.

On D3, an intraperitoneal injection was made of solutions of liposomes containing doxorubicin (DXR), or liposomes of DXR and quinine or DXR and cinchonine which were analogous to those which were used in c.2 and c.3, respectively, in the doses indicated.

Group 1: liposomes containing DXR and quinine in a dose of 0.5 mg of DXR/kg and 80 mg of quinine/kg.
Group 2: liposomes containing DXR and cinchonine in a dose of 0.5 mg of DXR/kg and 80 mg of cinchonine/kg.
Group 3: liposomes containing DXR (0.5 mg/kg)
Group 4: liposomes containing quinine (80 mg/kg)
Group 5: liposomes containing cinchonine (80 mg/kg)
Group 6: controls.

Each group consisted of 5 rats. They were sacrificed on D34, autopsies were performed, and the tumor nodules in each subject were weighed. The values listed below are the means obtained for the 5 subjects in each group.

Group 1: 2.0 g (± 1.0)
Group 2: 1.0 g (± 0.8)
Group 3: 2.0 g (± 0.6)
Group 4: 13.0 g (± 10.0)
Group 5: 4.6 g (± 3.8)
Group 6: 5.0 g (± 1.2)

This experiment confirms that substances such as quinine and cinchonine, associated with a cytotoxic agent such as doxorubicin within a liposome,

significantly inhibit the resistance of the carcinomatosis that was tested to anticancer agents.

Claims:

1. Therapeutic agent, especially one that is intended for the treatment of cancer tumors, comprising, associated in the same particular vehicle, a substance having a cytotoxic effect and at least one non-cytotoxic substance that inhibits multiple resistance to anticancer agents (MDR).

2. Therapeutic agent according to Claim 1, characterized by the fact that the particular vehicle is a microparticle, a microcapsule, a nanoparticle, or a nanocapsule.

3. Therapeutic agent according to Claim 1 or 2, characterized by the fact that the particular vehicle is a liposome.

4. Therapeutic agent according to one of Claims 1 through 3, characterized by the fact that the substance having a cytotoxic effect is chosen from the Vinca alkaloids, the anthracyclines, the epipodophyllotoxins, and the antitumor antibiotics.

5. Therapeutic agent according to one of Claims 1 through 4, characterized by the fact that the substance having a cytotoxic effect is chosen from vincristine, vinblastine, vindesine, vinorelbine, doxorubicin, deoxydoxorubicin, tetrahydropyranyladriamycin, epidoxorubicin, aclacinomycin, demethoxydaunorubicin, daunorubicin, *m*-AMSA, mitoxantrone, bisantrene, plicamycin, actinomycin D, puromycin, etoposide, teniposide, emetine, ethidium bromide, cytochalasin, colchicine, and Taxol®, or their acylated derivatives or esters.

6. Therapeutic agent according to one of Claims 1 through 5, characterized by the fact that the non-cytotoxic substance that inhibits multidrug resistance is chosen from amiodarone, quinine, quinidine, cinchonine, cinchonidine, verapamil, cyclosporine A, the cephalosporins, biperiden, lidocaine, chlorpromazine, pentazocine, promethazine, canrenoate potassium, amitriptyline, propranolol, demethoxyverapamil, diltiazem, thioridazine, trifluoperazine, chloroquine, sdb-ethylene diamine, reserpine, tamoxifen and toremifene, hydrocortisone, progesterone, salbutamol, and their acylated derivatives or esters.

7. Therapeutic agent according to one of Claims 1 through 6, characterized by the fact that the substance having a cytotoxic effect and the substance that inhibits multidrug resistance are both at least partly incorporated in the particular vehicle.

[*Translator's note: The French original of the following claim does indeed say "cytostatic" rather than "cytotoxic."*]

8. Therapeutic agent according to one of Claims 1 through 6, characterized by the fact that the substance having a cytostatic effect is found in the empty space of the particular vehicle, and the substance having an MDR-inhibiting effect is fixed to the membrane of the said vehicle.

9. Therapeutic agent according to one of Claims 1 through 6, characterized by the fact that the substance having an MDR-inhibiting effect is found in the empty space of the particular vehicle, and the substance having a cytotoxic effect is fixed to the membrane of the said vehicle.

10. Therapeutic agent according to one of Claims 1 through 9, characterized by the fact that it comprises, associated in the same liposome, mitoxantrone and quinine or cinchonine.

11. Therapeutic agent according to one of Claims 1 through 9, characterized by the fact that it comprises, associated in the same liposome, doxorubicin and quinine or cinchonine.

12. Pharmaceutical compound comprising, in a quantity that is sufficient to be effective, a therapeutic agent according to one of Claims 1 through 11 and an inert excipient, support, or diluent.

13. Pharmaceutical compound according to Claim 12, characterized by the fact that it is in the form of a suspension which can be administered via the parenteral route.

14. Pharmaceutical compound according to Claim 12, characterized by the fact that it is in the form of a gel which can be administered via the intraperitoneal route.

15. Process for preparing the compound according to Claim 12, characterized by the fact that it consists of associating the said cytotoxic substance, within the same particular vehicle, with at least one non-cytotoxic substance that inhibits multiple resistance to anticancer agents.

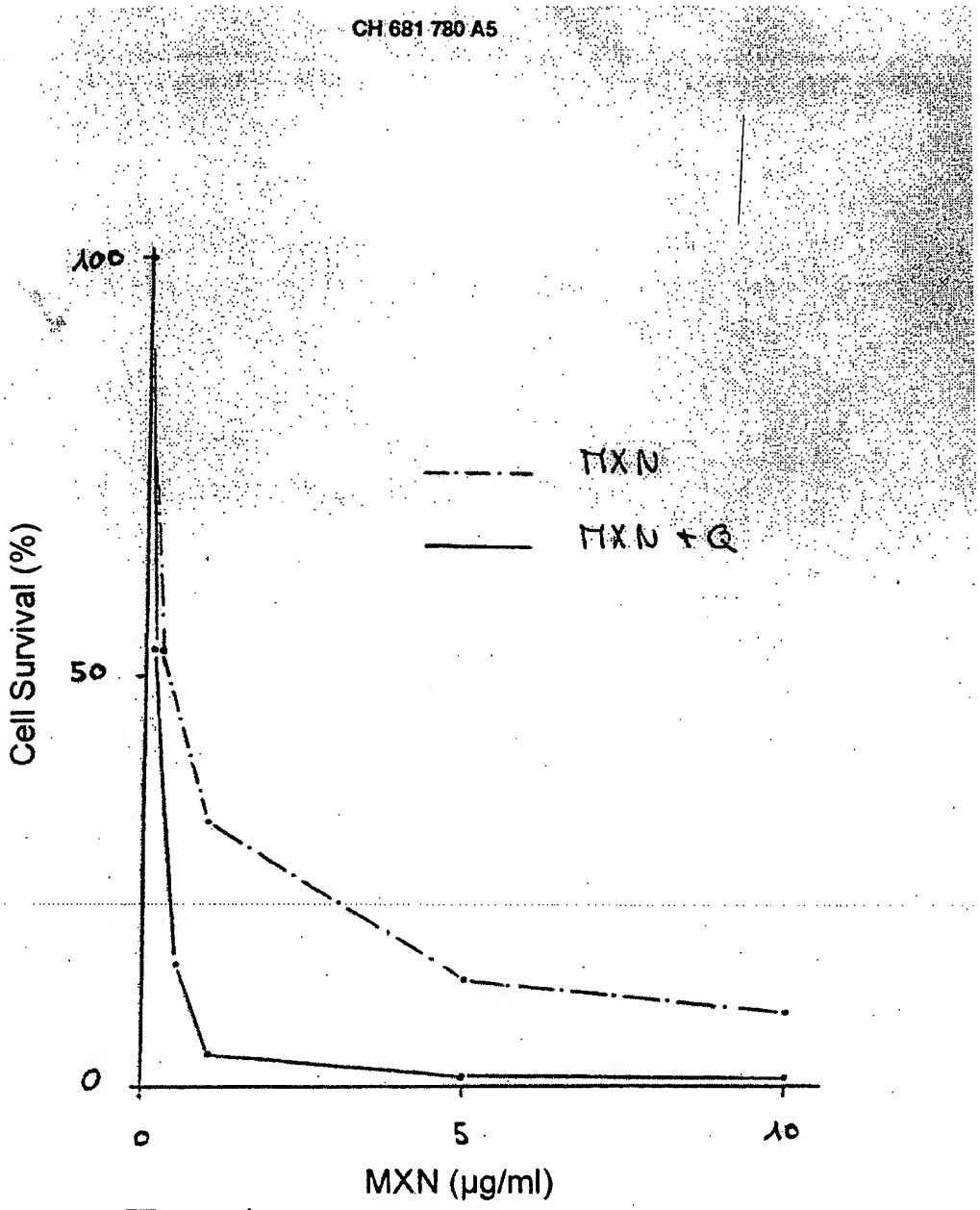

Fig. 1

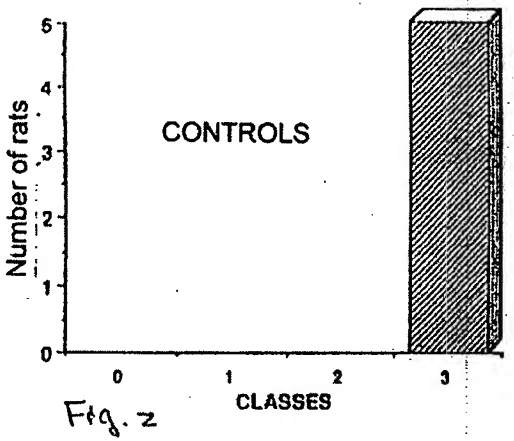

Fig. 2

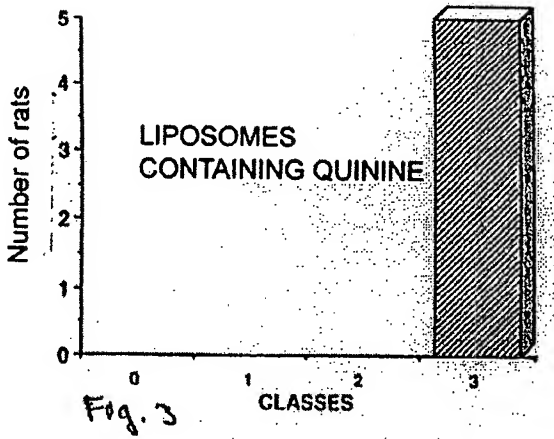

Fig. 3

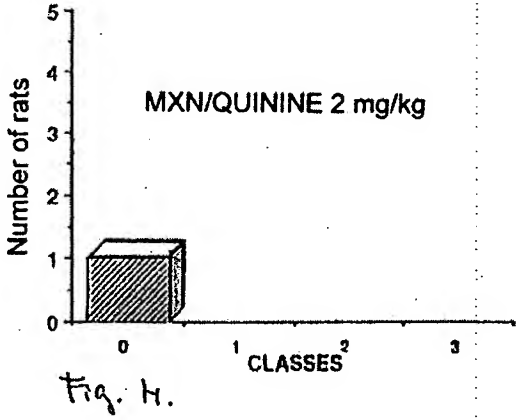

Fig. 4

LIPOSOME MXN/QUININE 2 mg/kg

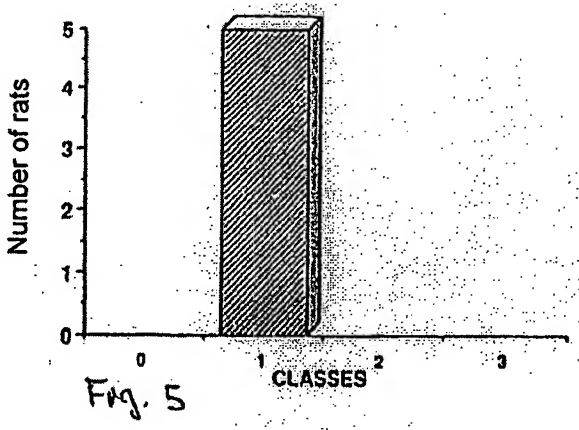

Fig. 5

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